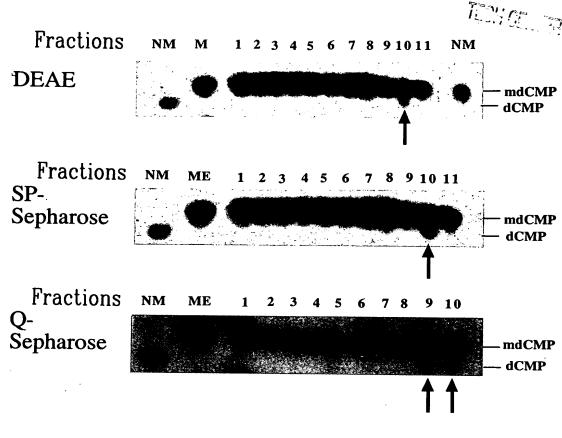
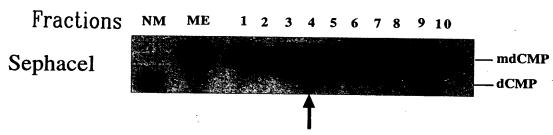


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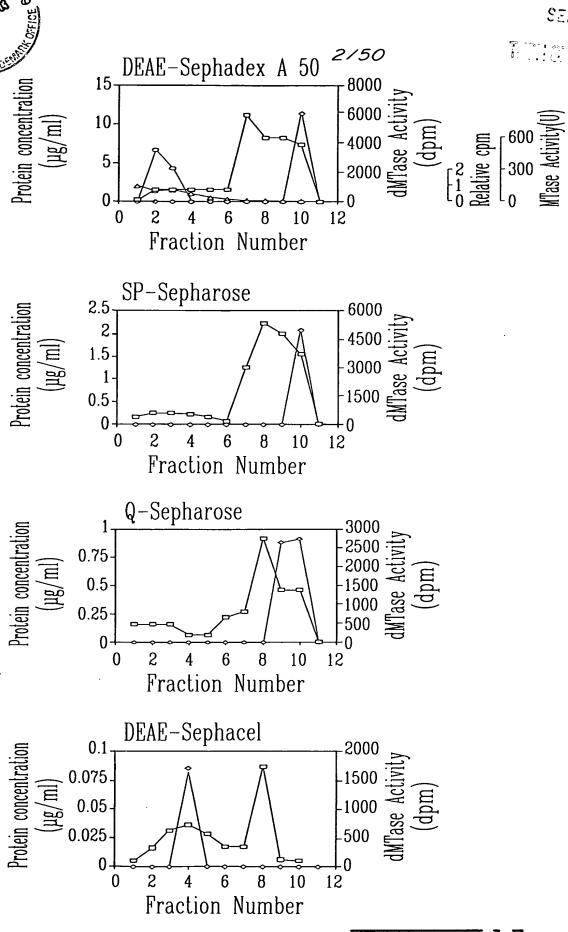






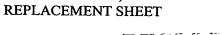
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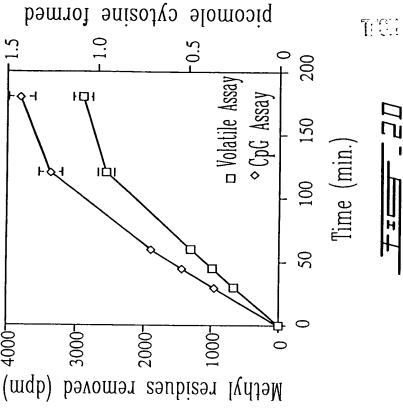
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75-7-3A	— mdCMP — dCMP	<u> </u>	- mdCMP	•
Proteinase K		7 <b>4</b> P		
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qqCTP mdCTP		Т		
Sephacel		ЧТ		
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DEAE-Sephadex S-Sephadex		nim 0£		
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WN	•	MN		



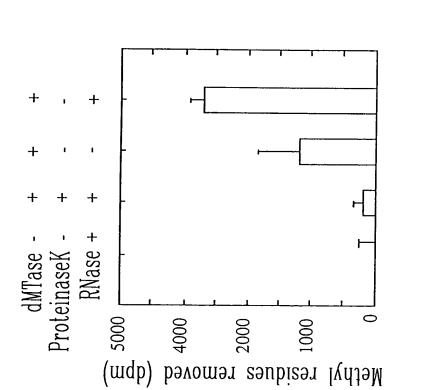
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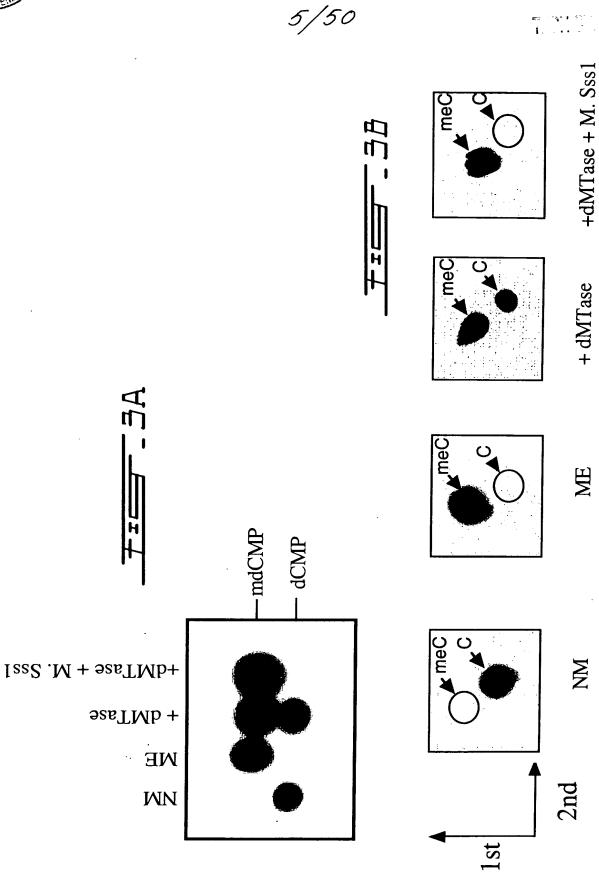


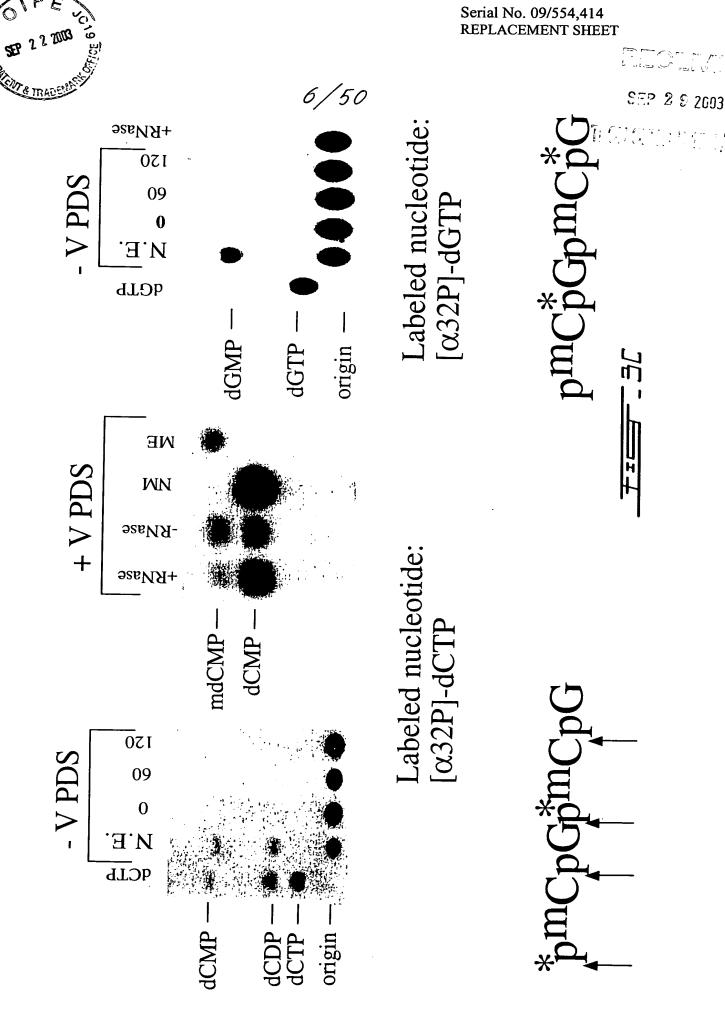
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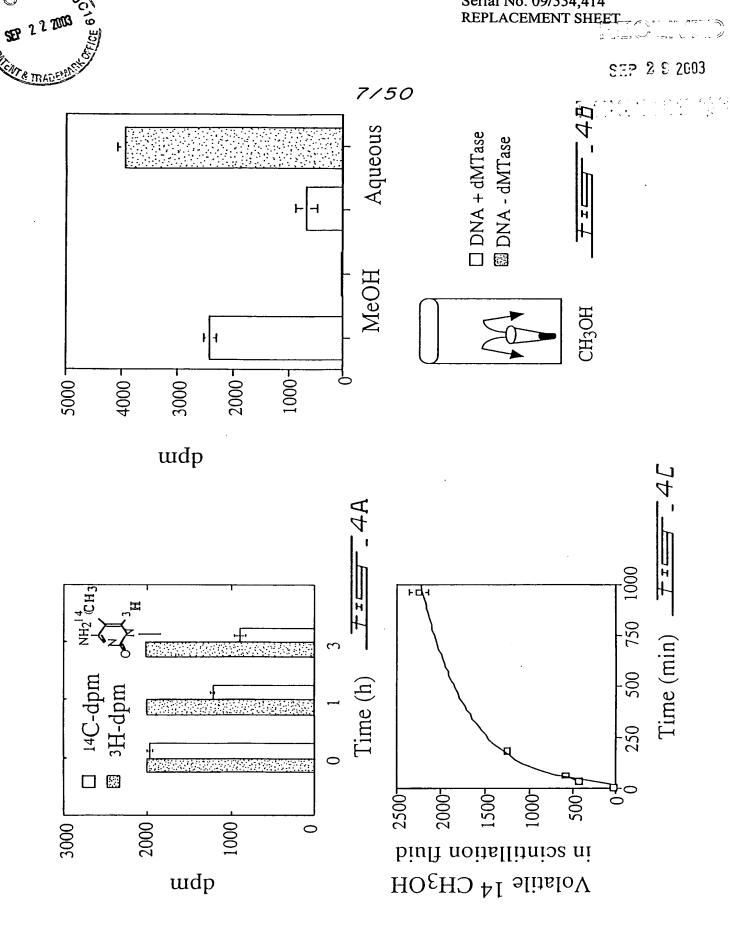








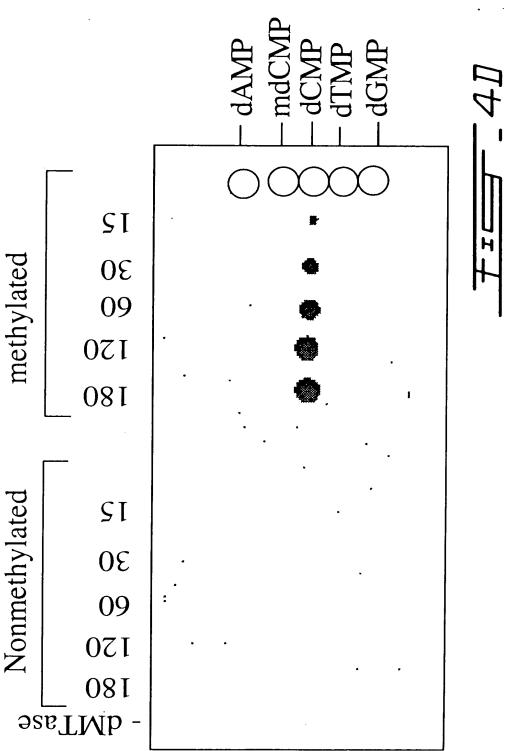






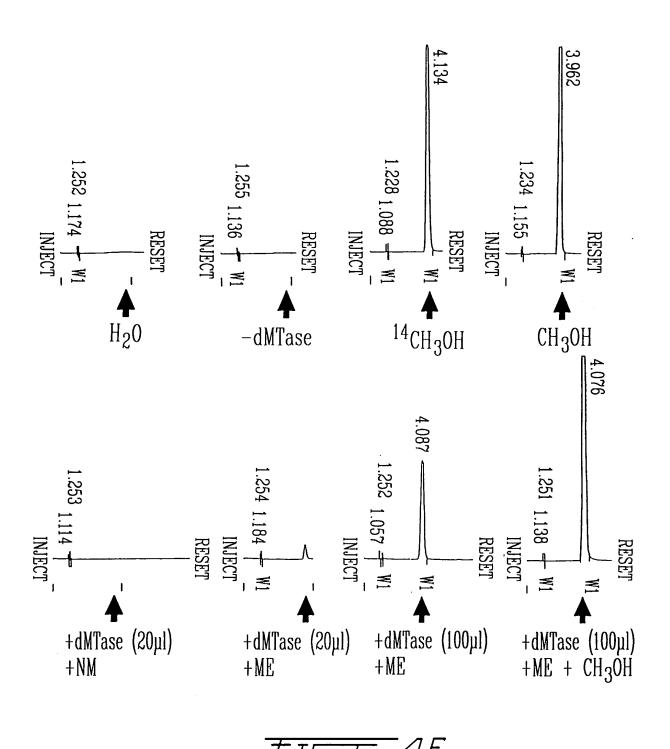
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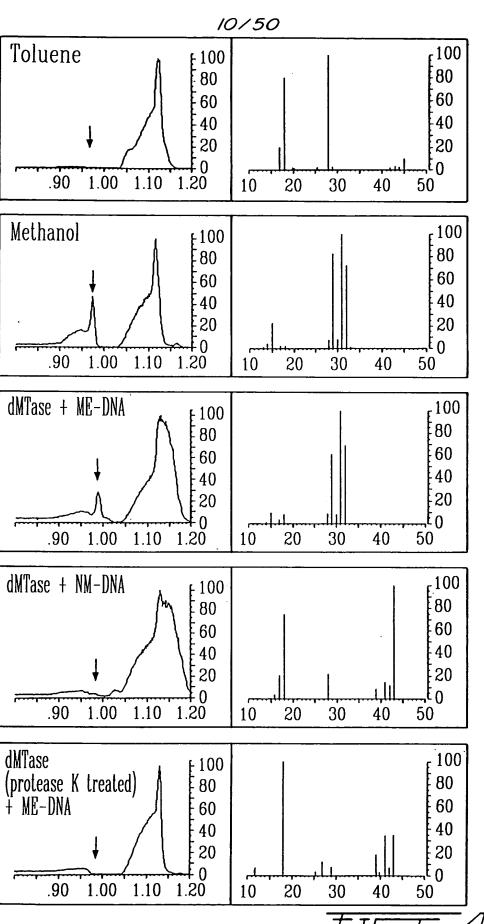


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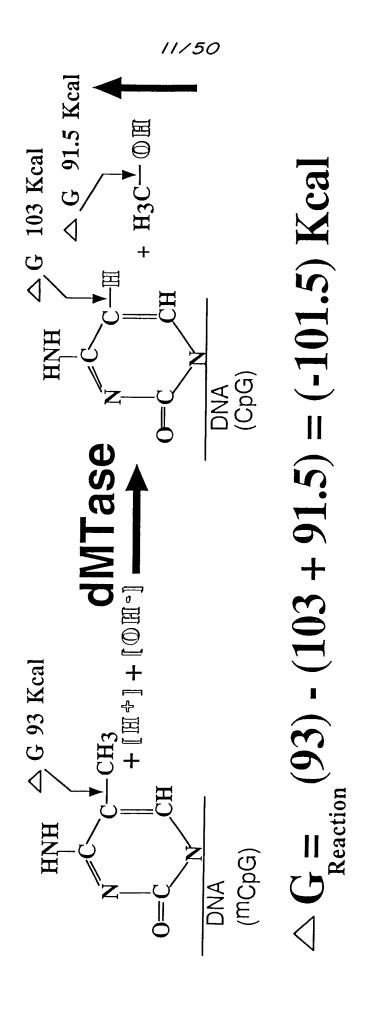
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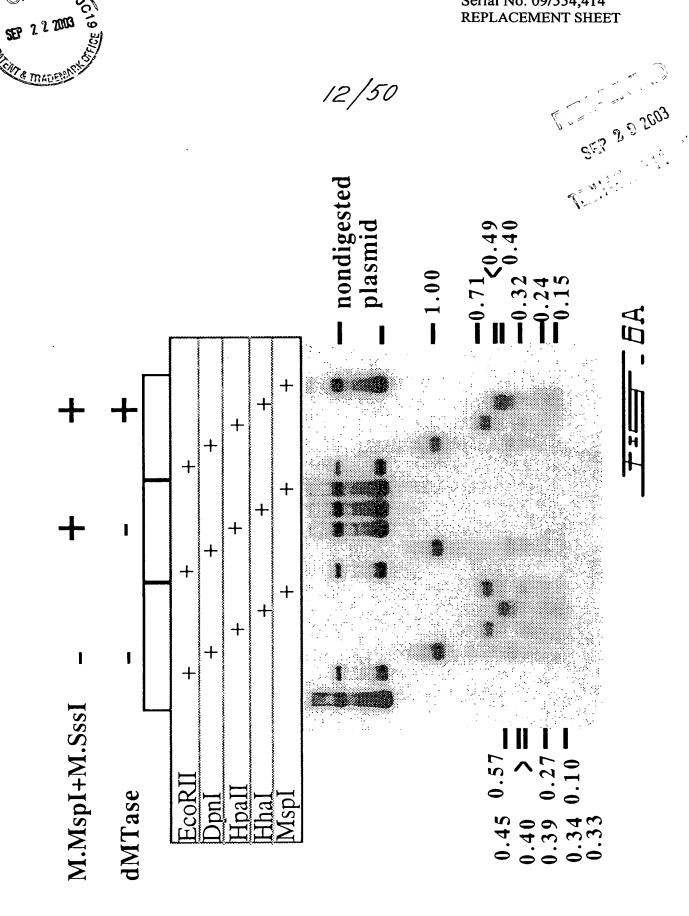


7



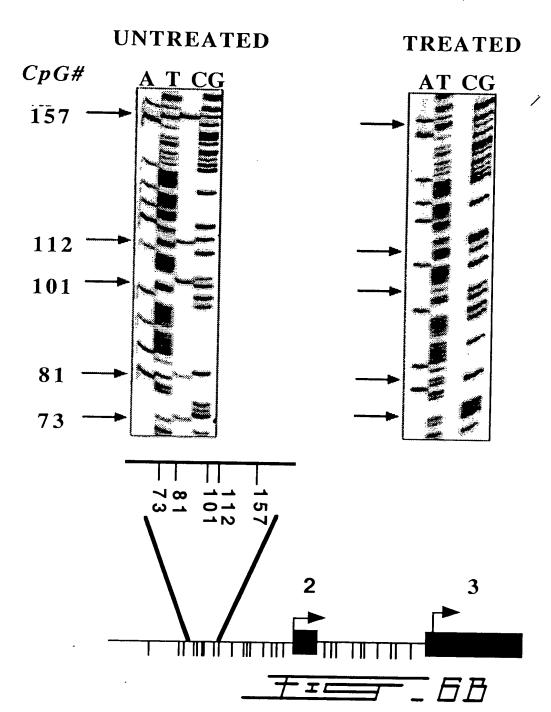


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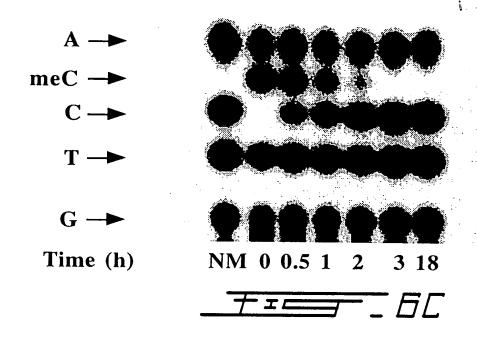


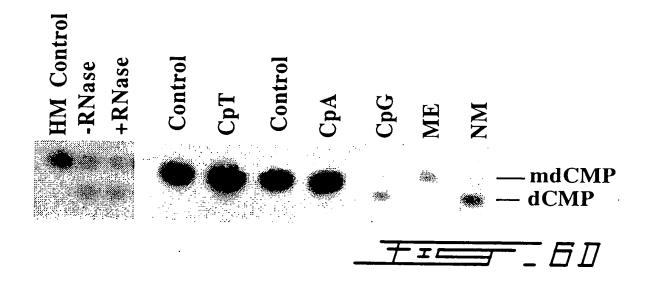


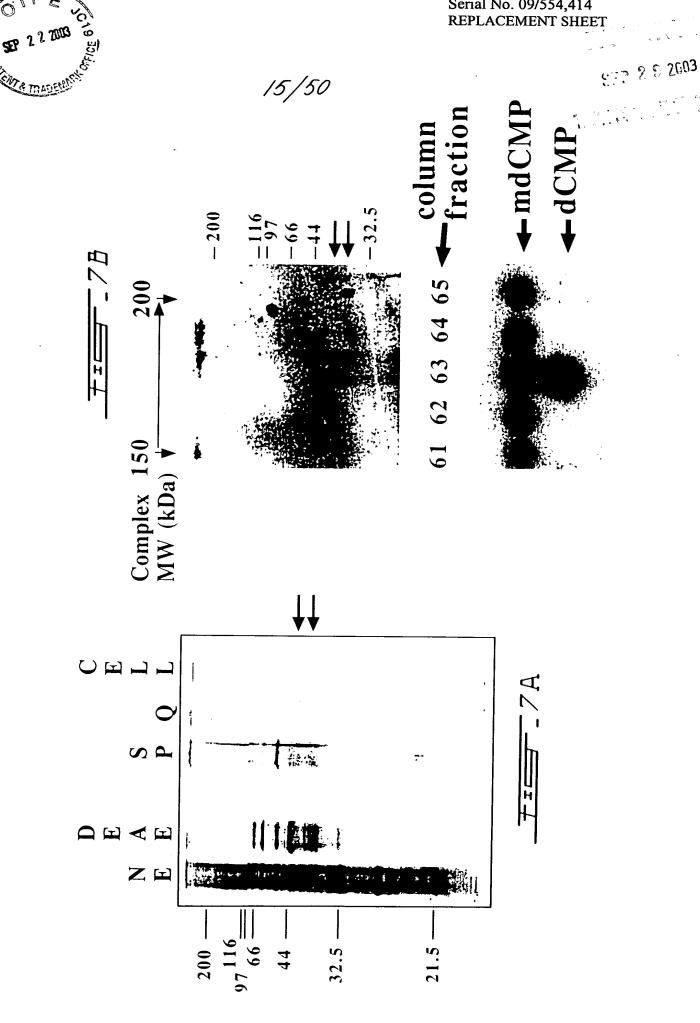




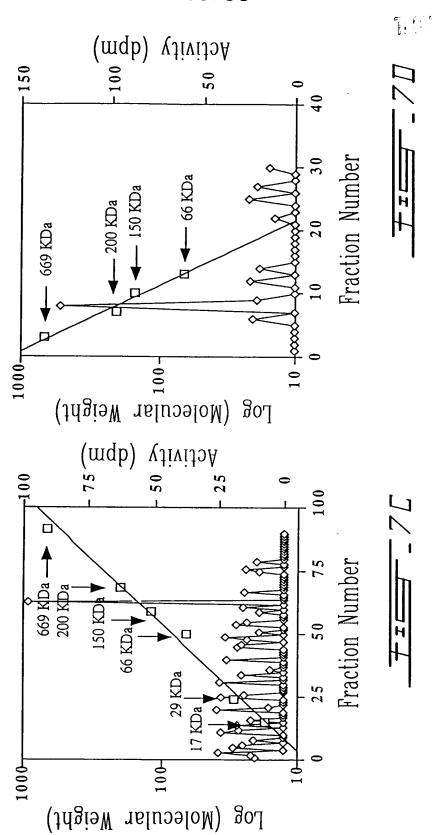
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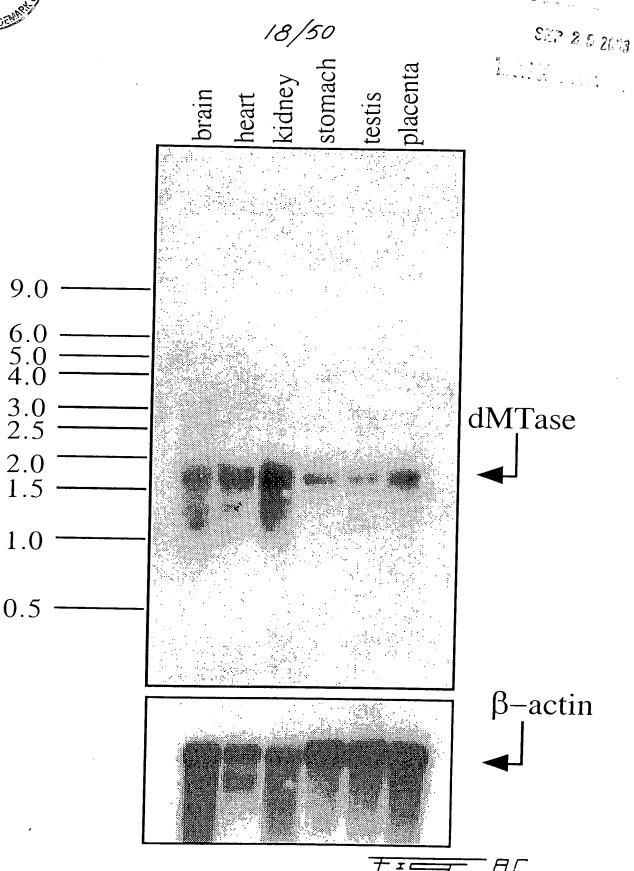
17/50

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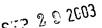
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40	80	120	160	200	240	262
MDCPALPPGW KKEEVIRKSG LSAGKSDVYY FSPSGKKFRS 40	KPOLARYLGN TVDLSSFDFR TGKMMPSKLQ KNKQRLRNDP 80	LNQNKGKPDL NTTLPIRQTA SIFKQPVTKV TNHPSNKVKS 120	DPQRMNEQPR QLFWEKRLQG LSASDVTEQI IKTMELPKGL	QGVGPGSNDE TLLSAVASAL HTSSAPITGQ VSAAVEKNPA 200	VWLNTSQPLC KAFIVTDEDI RKQEERVOOV RKILEDALMA 240	DILSRAADTE EMDIEMDSGD EA





caacattgcc ggaggaccta ccgggattcc tgagagagaa tagaacagta gcagcggcct cccccddcd gcccgctcta cgggcggcag gggggcggtg gaagtagaag cccgggccac gtccaagtgg ttgatctcag atcatcctag aggaggaagt acaaacagag catagcgctt ctctgtgcgc ggcagcgcgc ttacagaaga gacttgaata gagagtgcgg ggaagtagaa cggggccggg cccaggggac aaagtcacaa gggcgggtct gggggctgga ccgagtggcg ggcggcggcg ggatggaaga tactacttca ggaaatactg gatggccgcc aggaggccg gatgaggag cggctgggga ggaggaggg gggggccag taggggagga tggccgtggc aggaagtaa cggcagcggt actacacac gagcgatgtc aaggtacctg gcctagtaaa gggtaaacca accggtaacc 9999009999 agagagaaa agcgcagcgc ccatagagca attgcccggc gcggagacaa cdccccdddc gcccggagca ctcagttggc gggaaggcgc gaggagtatg gagaagada cggggagcgc gaagggaag gtgctggcaa tttcaaaca gaaagatgat atcaaaataa gccggggcca gagagracaa ggaagatgat ttacggaaga ggccggggcg ccccgagaag ggcgactccg ggcgtgcgca cggggccggg ggaggagat agaagcaagc gatcctctca acagcatcaa cctttcccgt aagaggatgg tctgggctaa ttcagaactg 5'gggggcgtgg agaggcggtg aattagacaa tgatgcttgc cccgggggga actdcdaaac aagggctcgg cccggtgagc tggcggcgac ggagccggtc taagaagttc cagttttgac aggadataga gaagcaggcg gggacgggga ggagagcggg gatccgaaaa

Human DNA demethylase cDNA-dMTase1 and predicted amino acid sequence





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SEQ ID NO:1



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DCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNT

**VDLSSFDFRTGKMMPSKLQKNKQRLRNDPLNQNKGKPDLNTTLPIRQTAS** 

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WLNTSQPLCKAFIVTDEDIRKQEERVQQVRKKLEEALMADILSRAADTEE

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I FKQPVTKVTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVTEQII

SEQ ID NO:2

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22/50

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Human DNA demethylase homologue-dMTase2 and predicted amino acid sequence"

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SEQ ID NO:3

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75- 9F

SEQ ID NO:4

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- <u>96</u> -

SP 22 THAT THE TRADE

Lipman-Pearson Protein Alignment Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

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Seq1(1>411)	Seq2(1>291)	Simi	Similarity	Gap	Gap	Consensus
human dMTase1 protein	human dMTase2 protein	protein	Index	Number	Lenath	l enath
(148>397)	(4>253)		76.0	0		) ) ひろり
(148>397)	(4>253)		76.0	0	0	250
v150 v160	v170 v1	v180	v190	v200	v210	! !
KRMDCPAL PPGMKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGVIVDLSSFDFRIGKMPSK	<b>JIRKSGLSAGKSD</b>	<b>NYYFSP</b>	SCHKFRSK	POLARYLG	NVDL SSFDFR	TGKMMPSK
KR : CPALP. GW.: EEV R: SGLSAG DV: Y: SPSGKKFRSKPOLARYLG.:: DLS: FDFRIGKM: SK	7 R:SGLSAGD	W:Y:SP	SCHAFTSIK	POLARYIG.	::DLS:FDFR	TGKM: SK
KRWECPALPQGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLARYLGGSMDLSTFDFRTGKMLMSK	PRRSGLSAGHRD	<b>WFYYSP</b>	GAKKERSK	POLARYLG	SMOLSTFDFR	TGKMLMSK
, 10	^20 ^30		~40	~20	09~	25/:
						50

^140 MNKSRQRVRYDSSNQVKGKPDLNTAL.PVRQTASIFKQPVIKITINHPSNKVKSDPQKAVDQPRQL.FWEKKL : OPROLEWEK: L LOKNIKORLRNDPLNONKGKPDLNTTT.PIROTASIFKOPVIKVINHPSNKVKSDPORMNBOPROLFWEKRI v280^130 ::K::QR:R D: NQ KGKPDLNT:LP:RQTASIFKQPVIK:TNHPSNKVKSDPQ: v270 ^120 v260^110 v250^100 v240<u>م</u> v230 \ 08√ v220

SEP 2 9 2573



SEP 2 9 2603

^210 QELSASDVIEDIIKIMELPKGLQGVGFGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVMLNTSQP GL:A D::E:::KIM:LPKGLQGVGPG..DETLLSA:ASALHTS: PITGQ:SAAVEKNP:VMINT:QP SCINAFDIAEELVKIMDLPKGLQGVGPGCIDEII.I.SAIASALHISIMPITGQLSAAVEKNPGVMINITQP v350 ^200 v340 ^190 V330 ^180 v320^170 v310 ^160 v300^150 v290

LCKAFIVIDEDIRKQEERVQQVRKKI.EEAI MADII SRAAD LCKAFWVIDEDIRKQEELVQQVRKRLEEALMADMLAHVEE LCKAF:VIDEDIRKQEE VQQVRK:LEEALMAD:L::..: ^240 V380 ^230 v370 ^220 v360

SEP 2 0 2003



## Mouse DNA demethylase-dMTase1 and predicted amino acid sequence

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SEQ ID NO:5



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KRMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYL

GNAVDLSSFDFRTGKMMPSKLQKNKQRLRNDPLNQNKGKPDLNTTLPIRQ

TASIFKQPVTKFTNHPSNKVKSDPQRMNEQPRQLFWEKRLQGLSASDVTE

**QIIKTMELPKGLQGVGPGSNDETLLSAVASALHTSSAPITGQVSAAVEKN** 

PAVWLNTSQPLCKAFIVTDEDIRKQEERVQQVRKKLEEALMADILSRAAD

TEEVDIDMDSGDEA

SEP 28 2003

SEQ ID NO:6

+z = 3



Mouse DNA demethylase-dMTase2 and predicted amino acid sequence

## 30/50

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~ <sup>©</sup> 20

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ctgctccggc

agggtttgga

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gaagtttctg

tatcatagga

cctgcctcct

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aggaggcagc

ccctgtgga

gctgcagact

ccaagtctgg

ggtgccctgc

gtgtagcaca

gccagagcga

aagagccgga

gccttcagcc

ctttccaaag

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gtgcagggag

caaggccttg gaaacacacc agtcactttc

cccaccagga

gggtcagcag

cccacactga

gaccagacta

caccttatgt

tggctatgcc

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cttcaataaa

aggagctggg

gaacttgtg

ctgatggtag

ccctaaggct

SEQ ID NO:7



RYLGGSMDLSTFDFRTGKMLMNKMNKSRQRVRYDSSNQVKGKPDLNTALP MERKRWECPALPQGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLA VRQTASIFKQPVTKITNHPSNKVKSDPQKAVDQPRQLFWEKKLSGLSAFD IAEELVRTMDLPKGLQGVGPGCTDETLLSAIASALHTSTLPITGQLSAAV EKNPGVWLNTAQPLCKAFMVTDDDIRKQEELVQQVRKRLEEALMADMLAH VEELARDGEAPLDKACAEEEEEEEEEEEEEFFFFV

SEQ ID NO:8

NB - FEET

SE JO TO SOLUTION OF THE PARTY OF THE PARTY

Lipman-Pearson Protein Alignment
Ktuple: 2; Gap Penalty: 4; Gap Lenoth Penalty:

Nuple: 4; Gap Penalty: 4; Gap Length Penalty: 12	∵ Gap Leng	orn Penalty: 12						
Seq1(1>414)	Seq2(1>285)	,	Similarity	Gap		Gap	Consensus	
mouse dMTase1 protein	mouse dN	mouse dMTase2 protein	Index	Number	Le	Length	Length	
(151>400)	(4>253)		75.2	0		0	250	
(151>400)	(4>253)		75.2	0		0	250	
v160	v170	v180	v190	v200	v210	V220		
KRMDCPAL.PPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNAVDLSSFDFRIGKMPSK	VIRKSGLS	AGKSDVYYF,	SPSGKKFR	SKPQLARYI	GNAVDL	SSFDFRIC	KMMPSK	
KR : CPALP.GW.: EEV R:SGLSAGDV:Y:SPSGKKFRSKPQLARYLG.::DLS:FDFRTGKM: :K	V R:SGIS	AG. DV:Y:	SPSGKKFR	SKPQLARYI	G.:.DL	S: FDFRIC	KM: :K	
KRWECPAL POGWEREEVPRRSGLSAGHRDVFYYSPSGKKFRSKPQLARYLGGSMDLSTFDFRTGKMLMNK	VPRRSGIS	AGHRDVFYY	SPSGKKFR	SKPQLARYI	GGSMDL	STFDFRIC	KMIMK	
`_10	^20	√30	~40	<sup>^</sup> 50		09~	~20	<b>.</b>

v290 ^140 MIKSRQRVRYDSSNQVKGKPDLNTAL.PVRQTASTFKQPVTKLTINHPSNKVKSDPQKAVDQPRQL.FWEKKL LOKNKORLRNDPI NONKGKPDI NTTI PIRQTASI FKOPVIKFIINHPSNKVKSDPORMNEO PROLFWEKRL ::K::QR:R D: NQ KGKPDLNT:LP:RQTASIFKQPVTK:TNHPSNKVKSDPQ: :QPRQLFWEK:L v280 ^130 v270 ^120 v260^110 v250^100 v240<u>م</u> v230\ 80 80

1 = 1 = 1 = 1 = 1 = 1 = 1



v360 QELSASDVIEQIIKIMELEKGLQGVGEGSNDETLLSAVASALHTSSAPITGQVSAAVEKNPAVMLNTSQP ^210 SGLSAFDIAEELVRIMDLPKGLQGVGPGCIDEITLSALASALHTSIT.PIIGQLSAAVEKNPGVMLNIAQP GLSA D::E::::TM:LPKGLQGVGPG..DETLLSA:ASALHTS: PITGQ:SAAVEKNP:VMLNT:QP v350 ^200 v340^190 v330 ^180 **v**320 ^170 v310 ^160  $v_{300}$ ^150

v390 v380 v370

LCKAFIVIDEDIRKQEERVQQVRKKLEEALMADII.SRAAD LCKAF:VID:DIRKQEE VQQVRK:LEEALMAD:L::...

^250 LCKAFWVIDDDIRKQEELVQQVRKRLEEALMADMLAHVEE ^240 ^230 ^220

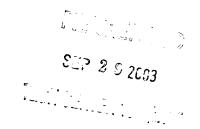
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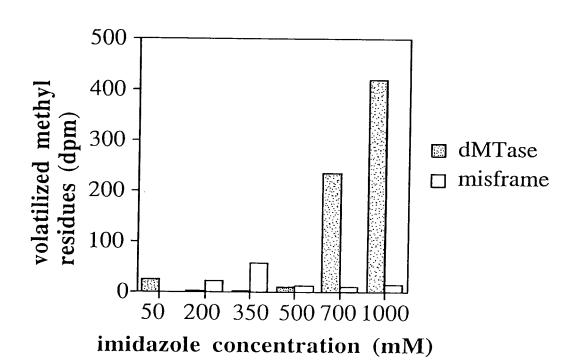
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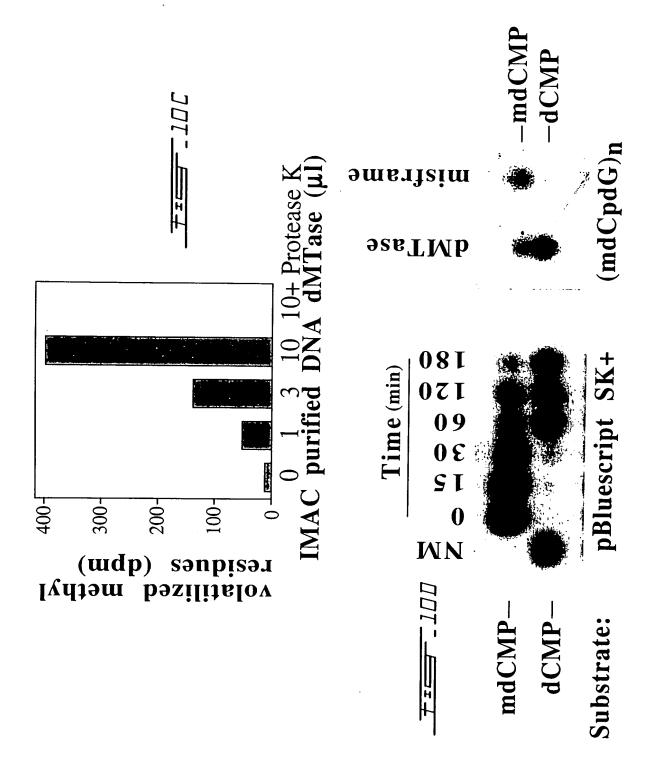
35/50 Not 1 T7 ATG (His)6 XpressEK MCS BGHpA 1.36 kb 66 45 29 pcDNA-His-dMTase misframe 98. ak MDBD Not 1 **PCMV** 





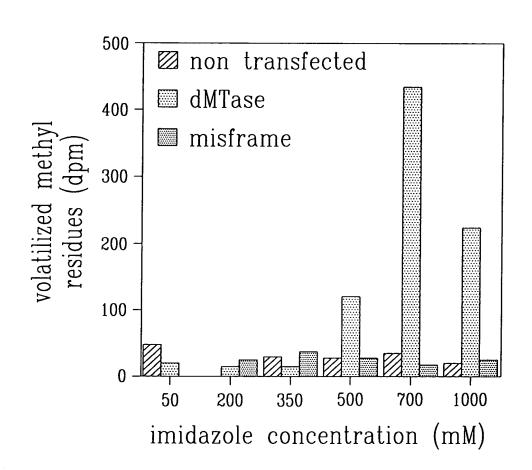






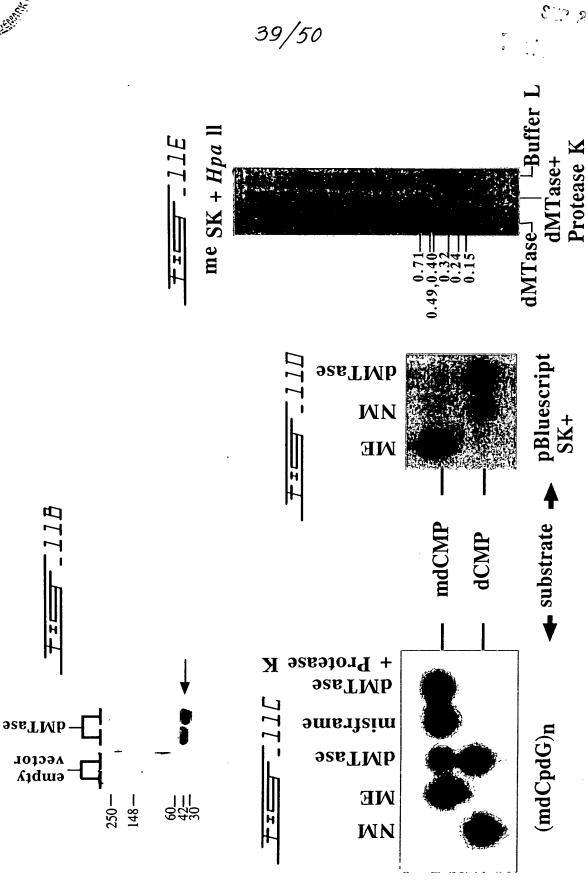


\$37 2 9 2.23 - • • • • • • • • • • • • • •

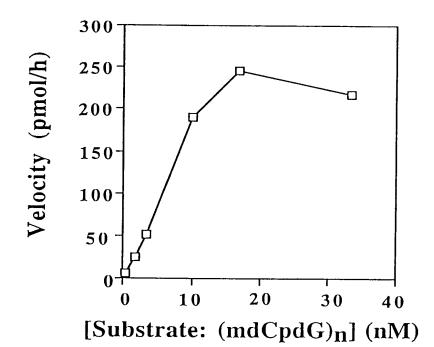


#==== 11A











- Origin

En Esqua

Transient dMTase

-Origin

-mdCMP O O O II- dCMP

100

05

01

methylated CpG DNA (ng)

100

05

10

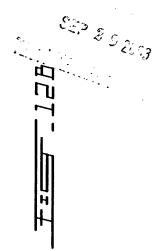
methylated CpG DNA (ng)

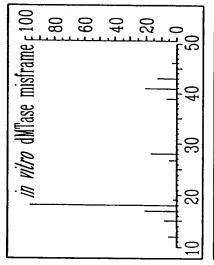
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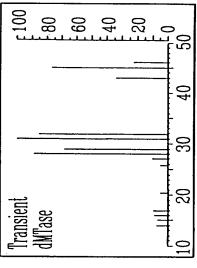
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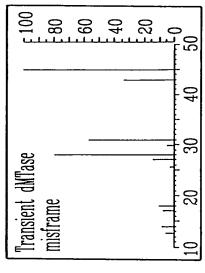
A549 dMTase

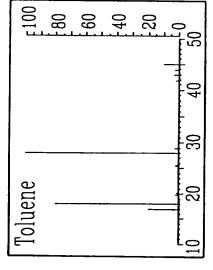


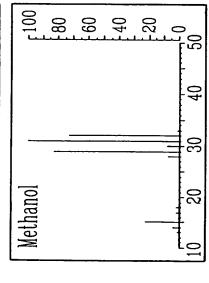


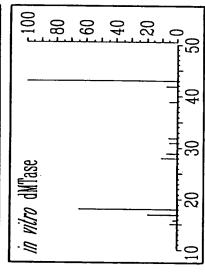




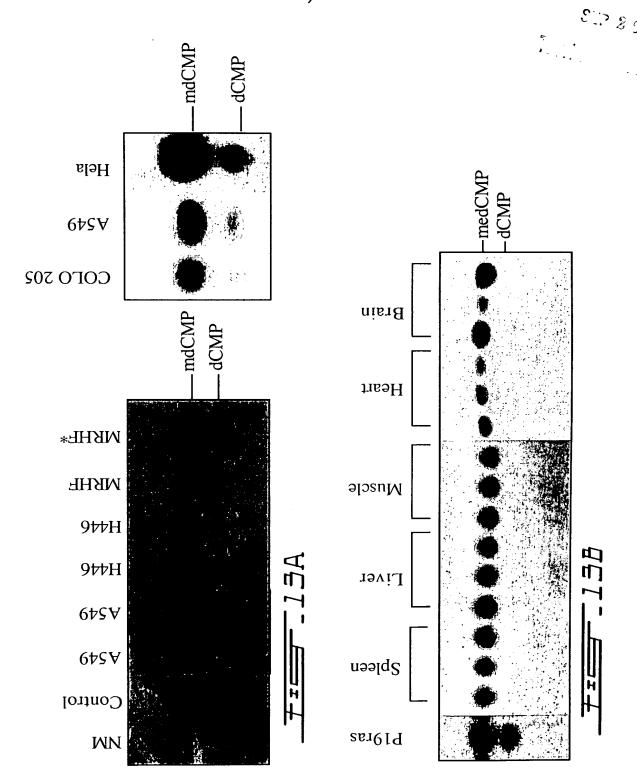




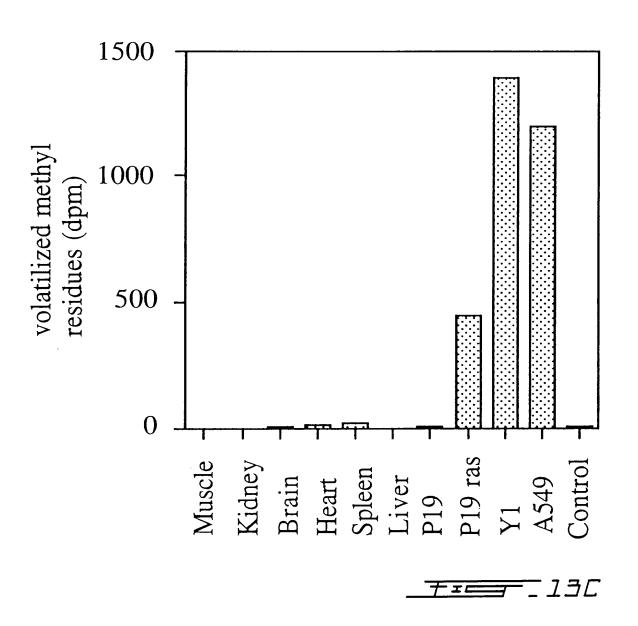




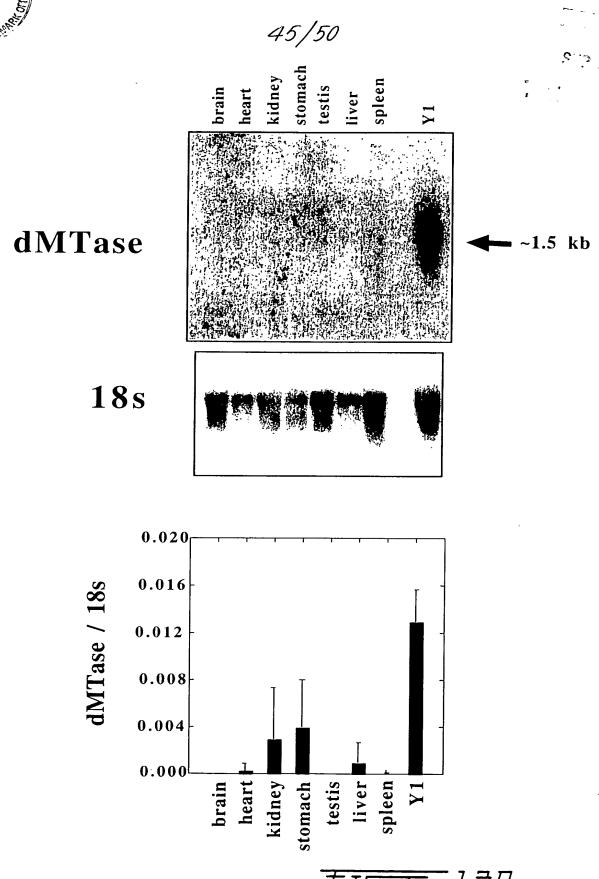


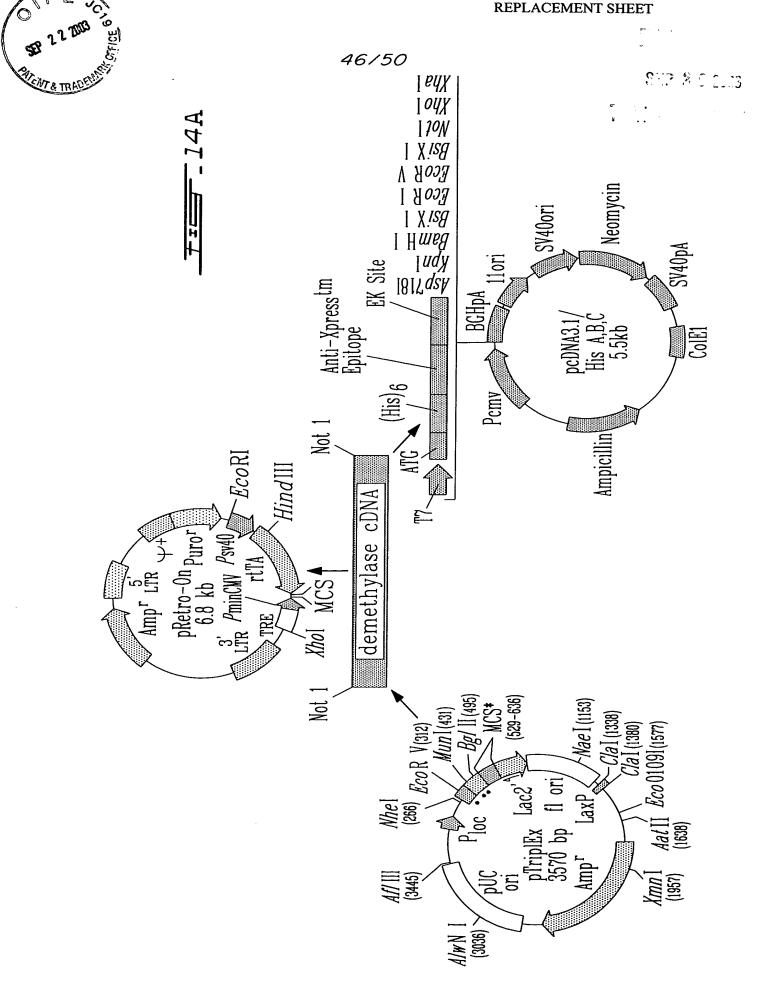




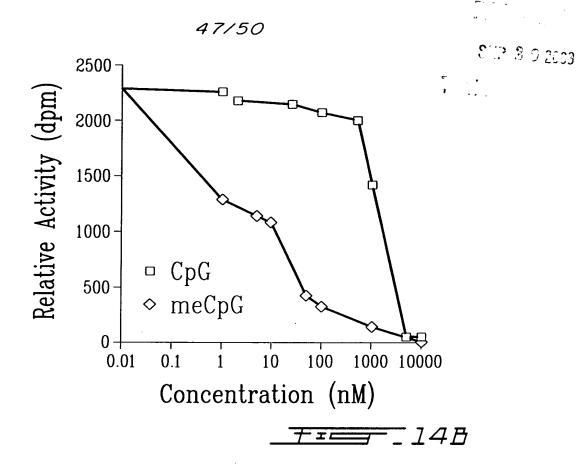


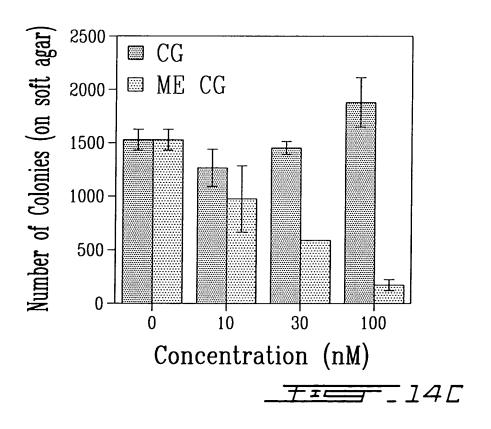






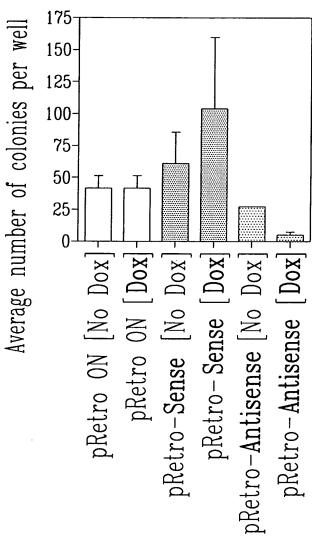


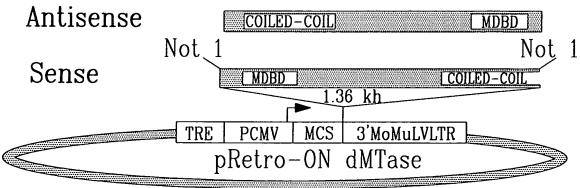




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